THE TENSION AT THE SURFACE OF MARINE EGGS, ESPECIALLY THOSE OF THE SEA URCHIN, ARBACIA

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Biologists frequently speak of the surface tension of cells, comparing their form, movements and division to phenomena connected with surface tension at oil-water interfaces. It seems unlikely that the tension is a true surface tension between non-misceable fluids, but the behavior of deformed spherical cells shows very clearly that a surface force exists which can best be referred to as the *" tension at the surface "* without implying either elastic tension or surface tension. Estimates of its magnitude have been made in different ways and until recently the values obtained have been relatively high, from 10 to 50 dynes per centimeter. I believe the tension is very much smaller than this in many cells.

In a recent paper (1931) I have described an approximation method for determining the tension at the surface of an unfertilized egg in sea water, from the centrifugal force necessary to pull the egg apart. In the worm, *Chætopterus pergamentaceus,* the forces necessary are small and the whole process can be observed and photographed in the microscope-centrifuge (Harvey and Loomis, 1930), whose maximum speed is 4000 R.P.M. The value obtained for *Chætopterus* was about one dyne per centimeter, which represents the *maximum* value, since all the assumptions made were such as to give a maximum. The true value is probably considerably less than this, but the fact that the surface forces are so low is a point of great interest.

The method does not allow us to decide whether this force is a true surface tension at a liquid-liquid interface or the elastic tension of a membrane, because we cannot tell whether or not the strain is independent of the stress. Micro-dissection studies (Chambers, 1921) indicate that the surface of marine eggs is surrounded by an actual consistent film variously spoken of as the " pellicle " or vitelline membrane,

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which lifts off and hardens to form the fertilization membrane of *Ar-bacia* eggs. Since a *thin* elastic film will take the same configuration under distortion as a surface showing true surface tension, and since the pellicle in the egg becomes discontinuous at the time of cell division or when the egg is fragmented by centrifuging, we may regard such a film as having the properties of a true surface (except for the relation between stress and strain), and on this basis calculate its tension by methods which at least give order of magnitude and a maximum value.

The argument for the *Arbacia* egg is somewhat different from that used in the case of *Chætopterus*, because *Arbacia* pulls apart in a quite different manner. In *Chætopterus* in sea water an oil spherule pulls away from the rest of the egg at 4000 R.P.M. (11 cm. radius), re-maining attached to the egg by a long stalk pulled out to many times the diameter. The picture is the same if the eggs are suspended in a sugar solution of the same density or of greater density, when the eggs float. In the latter case a yolk spherule is pulled away from the re-mainder of the egg, again with a long connecting stalk.

Unfertilized eggs of the sea urchin, *Arbacia punctulata,* cannot be rapidly pulled apart with this force but, at about 7000 R.P.M. (same radius), if suspended in a medium of the same density as the egg, they elongate, form dumb-bell shapes and in 4 minutes separate into a lighter and a heavier half of nearly the same size. The egg may be regarded as a sphere pulled into a cylinder with rounded ends by the buoyant force of the oil and the weight of the heavier yolk mass. In this process the surface area increases about 25 per cent.

It is well known that a cylinder of fluid becomes an unstable form when its length exceeds its circumference ($2\pi r$), *i.e.,* when its length is about three times its diameter (Lord Rayleigh, 1879). Under these circumstances it will divide into two. The surface tension (σ) around the circumference should then just balance the forces pulling the cylinder apart, $\sigma 2\pi r =$ forces stretching cylinder.

If we regard the egg as a sphere non-misceable with sea water, we can calculate its circumference when drawn into a cylinder with hem-ispherical ends whose length is π times its diameter. The breaking up into two spheres of such a form will only be delayed because of the viscosity of the sphere.

We wish to know the radius of a cylinder of height, *h,* with hem-ispheres at each end of radius, *r,* in which $h + 2r = 2\pi r$ and whose volume equals that of a sphere, the *Arbacia* egg, of diameter, *d.*

$$\text{Hence,} \qquad \pi r^2 h + 4/3\pi r^3 = 4/3\pi (d/2)^3$$
$$\text{But,} \qquad h = \pi 2r - 2r$$
$$\text{Substituting,} \quad \pi r^2 (\pi 2r - 2r) + 4/3\pi r^3 = 4/3\pi (d/2)^3$$
$$\text{or} \qquad 2\pi^2 r^3 - 2\pi r^3 + 4/3\pi r^3 = 4/3\pi (d/2)^3.$$

Since the average diameter of an *Arbacia egg* is 74 μ,

$$17.65r^3 = 212000 \; \mu^3$$
$$r = 23.0 \; \mu.$$

The forces pulling the egg apart are due to the weight of the heavy fragment (H) and the buoyancy of the light fragment (L), which can be determined from the volume of the fragments (V), their densities (ρ), and the density of the medium (ρ_M) which is equal to the density of the whole egg since the eggs are centrifuged in a medium of equal density.

Force (in dynes) $= V_H \, (\rho_H - \rho_M) + V_L \, (\rho_M - \rho_L) \times 980 \times C$, where $C =$ centrifugal force in terms of gravity.

Since the egg pulls into approximately equal parts, the density of the light fragment must be as much less than the medium as the density of the heavy fragment is greater than the medium.[1] If V_E is the volume of the egg, the whole relation therefore becomes:

$$2\pi r \sigma = V_E \, (\rho_M - \rho_L) 980 \times C. \tag{1}$$

It is only necessary to know the diameter of an egg, the density of the medium and that of the light fragment and the centrifugal force to divide the eggs.

The diameter of the *Arbacia* egg (74μ) gives a volume (V_E) of 2.12×10^{-7} cm.³ To get the density of the eggs (without jelly) they must be suspended in a medium of the same osmotic pressure as well as the same density. Lucké has found that cane sugar of .95 molal concentration (342 grams cane sugar added to 1 liter water) causes neither swelling nor shrinking of *Arbacia* eggs.[2] I find that when centrifuged in one part of sea water and 3 parts of .95 M cane sugar, some

[1] Dr. Balduin Lucké, in the course of some experiments on the osmotic properties of yolk and clear halves, has measured the volumes in cubic micra and compared the sum of these volumes with the volume of the original egg. Each figure is the mean of 50 cells and must be multiplied by 100.

Date	Control egg A	Colorless half B	Yolk half C	Sum of B and C
Aug. 7........	1895	1013	939	1952
" 8........	2088	˙1185	932	2117
" 13........	1954	1129	814	1943
" 14........	1905	983	904	1887
" 15........	2030	1120	945	2065

The yolk half is somewhat smaller than the colorless half, averaging around 11 per cent smaller.

[2] Private communication. See also Lucké, 1931. *Biol. Bull.*, **60**: 75.

lots of eggs float, most sink and some remain suspended even under high centrifugal forces. This mixture has a density of 1.081 at 23° C.[3] In one part sea water and four parts .95 M cane sugar the eggs of most females float; others sink very slowly. Its density is 1.085 at 23° C. We may consider the density of the medium (ρ_M) and the egg to be 1.083. I believe Heilbrunn's (1926) value of 1.0485 (12.5 per cent sugar) and 1.0656 (16.5 per cent sugar) for different lots of eggs are too low, because he suspended the eggs in pure sugar solutions which were hypotonic and volume changes must have occurred. However, the density of different lots of eggs does vary considerably. Eggs with jelly have a density of about 1.090, whereas without jelly the density is about 1.083 to 1.084.

To obtain the density of the light fragments (ρ_L), the eggs (*without jelly*) are centrifuged in the sugar-sea water mixture of the same density and the light fragments transferred to other mixtures of sugar and sea water. They mostly float in 10 parts of sea water to 20 parts .95 M sugar, whose density is 1.076, whereas they all sink in 12 parts sea water to 20 parts .95 M sugar, whose density is 1.073. We may therefore consider their density to be 1.075 and $\rho_M - \rho_L = .008$.

The sugar-sea water mixture of the same density as the egg is not toxic. Although eggs will not fertilize in the sugar solution, they can be fertilized and develop normally when removed to sea water after an immersion of five hours.

In determining the centrifugal force the time factor is an important consideration. Part of the time is involved in the separation of granules of different density within the eggs. Only when this happens do the stretching forces appear. Part of the time is connected with the slow pinching of the egg in two. Even when an egg has assumed a prolate spheroid shape, recovery of the spherical form is very slow, a matter of many minutes. Dumb-bell-shaped eggs do not pinch in two after the centrifugal force is removed but remain dumb-bells for many minutes, gradually becoming spherical again (after 40 minutes). There is also considerable variation in the ease with which eggs can be pulled apart. Eggs from some females fragment at 122 r.p.s. in 4 minutes, others do not but will fragment in 12 minutes. Eggs which fragment in 4 minutes at 122 r.p.s. are not pulled apart in 4 minutes at 112 r.p.s., but fragment in 12 minutes. The same eggs centrifuged for 20 minutes at 100 r.p.s. do not fragment but do in 24 minutes. The eggs of some females pulled apart at 60 r.p.s. in 30 minutes, but not at 50 r.p.s. in 90 minutes.

[3] Densities were determined with a hydrometer calibrated for 15° C./15° C., reading to the third decimal place. The temperature correction will be small.

EDITOR'S NOTE:

The equation on page 277 of the article by E. N. Harvey in the December, 1931, issue should be corrected to read as follows:

$$\sigma = \frac{2.12 \times 10^{-7} \times .8 \times 10^{-2} \times 10^{3} \times 1.6 \times 10^{3}}{6.28 \times 23 \times 10^{-4}} = 0.19 \text{ dynes per cm.}$$

The result is very much lower than the 10 to 25 dynes per centimeter observed by Vlès (1926) for the egg of another sea urchin, probably *Paracentrotus*.

Should the eggs rest on a surface so that only the buoyant force of the light half is operative, the value will be about one-half of the above. The stretching forces must act against not only a tension at the surface, but viscous forces of the egg as well, which again will lower the figure. Finally, the calculation is based on the view that the tension is a true surface tension. If the elastic tension of a pellicle is involved, we are observing its breaking strength and its tension must be considerably less for a given stretch. It must be emphasized that 0.2 dyne per centimeter is not a very accurate value, but a maximum one, and again illustrates the very low tension at the surface of eggs presumably surrounded with a pellicle. The question arises as how general this order of magnitude is for other marine eggs.

Dr. H. K. Hartline has pointed out to me that fluid spheres from which a small oil spherule is drawn out should become unstable when the neck connecting oil spherule with the sphere has the same diameter as the spherule. The spherule will then pinch off as a separate drop. In this case the surface tension around the circumference of the oil spherule should counterbalance the buoyant force of the oil. In the case of *Chætopterus* eggs, the separation of the oil spherule occurs only after a long stalk has been pulled out and the buoyant force of the oil was regarded as counterbalancing the tension around the circumference of the stalk, $9\,\mu$ in diameter (Harvey, 1931). If the circumference of the oil spherule is considered, $34\,\mu$ in diameter, the value for the tension at the egg surface comes out about one-quarter of 1.32 dynes, or .33 dynes per centimeter.

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If eggs which have been pulled into dumb-bell form at 120 r.p.s. are observed in the microscope centrifuge at 60 r.p.s., a few pull apart into two spheres. The connecting strand between the spheres does not become long and fine as in the pulling off of an oil spherule in *Chætopterus,* but the break can be observed to occur when the length is about three times the diameter of the resultant half egg.

Since the centrifugal force (C) in terms of gravity is given by $C = .443n^2$, for 11 cm. radius, where $n =$ revolutions per second, C is 6380 for 120, 5560 for 112, 4430 for 100, and 1595 for 60 r.p.s.

Selecting a speed of 60 r.p.s., and inserting in (1), we have:

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